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A/PROV

Docket Number:	A080P

PROVISIONAL APPLICATION FOR PATENT COVER SHEET (Large Entity)

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

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TITLE OF THE INVENTION (280 characters max)

BAFF RECEPTOR

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☒ Specification — *Number of Pages* 31

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☒ Other (specify) — 1 abstract

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

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Respectfully submitted,

SIGNATURE _____ DATE August 17, 1999

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PTO/SB/16 (8-98)

PROVISIONAL APPLICATION FOR PATENT COVER SHEET (Large Entity)

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APPLICATION

FOR

UNITED STATES PATENT

BAFF RECEPTOR

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FIELD OF THE INVENTION

The present invention relates to the use of a receptor to BAFF, a β-cell activating factor belonging to the Tumor Necrosis Family, and its blocking agents to either stimulate or inhibit the expression of B-cells and immunoglobulins. This receptor may have anti-cancer and/or immunoregulatory applications as well as uses for the treatment of immunosuppressive disorders such as HIV. In addition, the receptor and its blocking agents may play a role in the development of hypertension and its related disorders. Furthermore, cells transfected with the gene for this receptor may be used in gene therapy to treat tumors, lymphomas, autoimmune diseases or inherited genetic disorders involving B-cells. Blocking agents, such as recombinant variants or antibodies specific to the receptor, may have immmunoregulatory applications as well. Use of the receptor to BAFF as a B-cell stimulator for immune suppressed diseases including for example uses for patients undergoing organ transplantation (ie bone marrow transplant) as well as recovering from cancer treatments to stimulate production of B-cells are contemplated. Use of the receptor to BAFF as an adjuvant and or costimulator to boast and or restore B cells levels to approximate normal levels are also contemplated.

BACKGROUND OF THE INVENTION

The present invention relates to a novel receptor in the TNF family. A novel receptor has been identified, BAFF-R.

The TNF family consists of pairs of ligands and their specific receptors referred to as TNF family ligands and TNF family receptors (Bazzoni and Beutler, 1996). The family is involved in the regulation of the immune system and possibly other non-immunological systems. The regulation is often at a "master switch" level such that TNF family signaling can result in a large number of subsequent events best typified by TNF. TNF can initiate the general protective inflammatory response of an organism to foreign invasion that involves the altered display of adhesion molecules involved in cell trafficking, chemokine production to drive specific cells into specific compartments and the priming of various effector cells. As such, the regulation of these pathways has clinical potential.

Induction of various cellular responses mediated by such TNF family cytokines is believed to be initiated by their binding to specific cell receptors. At least two distinct TNF receptors of approximately 55 kDa (TNFR1) and 75 kDa (TNFR2) have been identified [Hohman et al., J. Biol. Chem. 264: 14927-14934 (1989) and Brockhaus et al., PNAS, 87: 3127-3131 (1990)]. Extensive polymorphisms have been associated with both TNF receptor genes. Both TNFRs share the typical structure of cell surface receptors including extracellular, transmembrane and intracellular domains. The extracellular portion of type 1 and type 2 TNFRs contains a repetitive amino acid sequence pattern of four cysteine rich domains (CDRs). A similar repetitive pattern if CDRs exist in several other cell surface proteins, including p75 nerve growth factor receptor, the B-cell antigen CD40 amongst others.

The receptors are powerful tools to elucidate biological pathways via their easy conversion to immunoglobulin fusion proteins. These dimeric soluble receptor forms are good inhibitors of events mediated by either secreted or surface bound ligands. By binding to these ligands they prevent the ligand from interacting with cell associated receptors that can signal. Not only are these receptor-Ig fusion proteins useful in an experimental sense, but they have been successfully used clinically in the case of TNF-R-Ig to treat inflammatory bowel disease, rheumatoid arthritis and the acute clinical syndrome accompanying OKT3 administration (Eason et al., 1996; Feldmann et al., 1996; van Dullemen et al., 1995). One can envision that manipulation of the many events mediated by signaling through the TNF family of receptors will have wide application in the treatment of immune based diseases and also the wide range of human diseases that have pathological sequelae due to immune system involvement. A soluble form of a recently described receptor, osteoprotegerin, can block the loss of bone mass and, therefore, the events controlled by TNF family receptor signaling are not necessarily limited to immune system regulation. Antibodies to the receptor can block ligand binding and hence can also have clinical application. Such antibodies are often very long-lived and may have advantages over soluble receptor-Ig fusion proteins which have shorter blood half-lives.

While inhibition of the receptor mediated pathway represents the most exploited therapeutic application of these receptors, originally it was the activation of the TNF receptors that showed clinical promise (Aggarwal and Natarajan, 1996). Activation of the

TNF receptors can initiate cell death in the target cell and hence the application to tumors was and still is attractive (Eggermont et al., 1996). The receptor can be activated either by administration of the ligand, i.e. the natural pathway or some antibodies that can crosslink the receptor are also potent agonists. Antibodies would have an advantage in oncology since they can persist in the blood for long periods whereas the ligands generally have short lifespans in the blood. As many of these receptors may be expressed more selectively in tumors or they may only signal cell death or differentiation in tumors, agonist antibodies could be good weapons in the treatment of cancer. Likewise, many positive immunological events are mediated via the TNF family receptors, e.g. host inflammatory reactions, antibody production etc. and therefore agonistic antibodies could have beneficial effects in other, non-oncological applications.

Paradoxically, the inhibition of a pathway may have clinical benefit in the treatment of tumors. For example the Fas ligand is expressed by some tumors and this expression can lead to the death of Fas positive lymphocytes thus facilitating the ability of the tumor to evade the immune system. In this case, inhibition of the Fas system could then allow the immune system to react to the tumor in other ways now that access is possible (Green and Ware, 1997).

SUMMARY OF THE INVENTION

Applicants have identified a cDNA clone that encodes a polypeptide, designated in the present application as "BAFF-R", that binds the tumor necrosis factor, BAFF, a B-cell activating factor belonging to the Tumor Necrosis Family. BAFF is the same molecule previously described in WO/9912964, which is incorporated by reference herein.

In one embodiment, the invention provides methods of using BAFF-R. Included in such methods are methods of stimulating B-cell growth, dendritic cell-induced B-cell growth and maturation or immunoglobulin production in an animal using BAFF-R polypeptide or co-stimulating B-cell growth, dendritic cell-induced B-cell growth and maturation or immunoglobulin production in an animal using BAFF-R polypeptide and an anti-T antibody, a CD40 ligand or an anti-CD40 ligand. Also included are methods of

inhibiting B-cell growth, dendritic cell-induced B-cell growth and maturation or immunoglobulin production in an animal using BAFF-R polypeptide.

In another embodiment, the invention provides methods of using BAFF-R in the treatment of autoimmune diseases, hypertension, cardiovascular disorders, renal disorders, B-cell lympho-proliferate disorders, immunosuppressive diseases, organ transplantation, inflammation, and HIV. Also included are methods of using agents for treating, suppressing or altering an immune response involving a signaling pathway between BAFF-R and its ligand.

In one embodiment, the invention provides pharmaceutical compositions comprising a BAFF-R polypeptide and a pharmaceutically acceptable excipient.

In another embodiment, the invention provides chimeric molecules comprising BAFF-R polypeptide fused to a heterologous polypeptide or amino acid sequence. An example of such a chimeric molecule comprises a BAFF-R fused to a Fc region of an immunoglobulin or an epitope tag sequence.

In another embodiment, the invention provides an antibody that specifically binds to a BAFF-R polypeptide. Optionally, the antibody is a monoclonal antibody.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleic acid sequence (SEQ ID NO:2) of a cDNA for human BAFF-R and its derived amino acid sequence (SEQ ID NO:1). Potential start of translation at either nucleic acid residue 219 or 228; cysteine rich domain (CRD) at nucleic acid residues 240-341 of SEQ ID NO: 2 (amino acid residues 8-41 of SEQ ID NO:1); and potential transmembrane region at nucleic acid residues 375-459 of SEQ ID NO:2.

Figure 2 shows the nucleic acid sequence (SEQ ID NO: 4) and its derived amino acid sequence (SEQ ID NO:3) of pJST538, a plasmid encoding BAFF-R-Fc: nucleic acid residues 1-69, murine IgG-kappa signal sequence; nucleic acid residues 70-222, BAFF-R(nucleic acid residues1-153); nucleic acid residues 223-906, human IgG.

Figure 3 shows the nucleic acid sequence of pJST535, a plasmid encoding a full length human BAFF-R and its derived amino acid sequence.

Figure 4 shows a structure comparison between TNF-R55 and BAFF-R.

Figure 5 shows 293EBNA cells transfected with either (a)CH269 (1.0ug) or (b)pJST535 (0.1ug), the plasmid expressing full length BAFF-R, and stained with 0.5ug/ml flag-hBAFF in the plate assay format.

Figure 6(a) shows FACS overlay of 293EBNA transfected with pJST535 and stained as follows: no ligand (black histogram), 1ug/ml flag-hCD40L (pink) or flag-hBAFF (green). All samples were then stained with anti-flag M2 followed by donkey anti-mouse IgG as described in methods in Example 2.

Figure 6(b) shows FACS histograms with statistics of same experiment. Staining is as follows: (1)unstained, (2) 7AAD only, (3) 2nd step and 7AAD only, (4) 9ug/ml flag-hBAFF, (5) 3ug/ml flag-hBAFF , (6) 1ug/ml flag-hBAFF, (7) 0.33ug/ml flag-hBAFF, (8) 0.11 ug/ml flag-hBAFF, (9)flag-hCD40L 1ug/ml.

Figure 7 shows Immunoprecipitations with BAFF-R-Fc as described in methods in Example 4. Molecular weight standards in kDa are as labeled to the left of the figure. Lane (1) 12.5 ng flag-hTWEAK, (2) 12.5 ng flag-hBAFF, (3) immunoprecipitation of flag-hBAFF by 0.5ml BAFF-R-Fc conditioned media, (4) immunoprecipitation of flag-hTWEAK by 0.5ml BAFF-R-Fc conditioned media, (5) immunoprecipitation of no ligand by 0.5ml BAFF-R-Fc conditioned media, (6) immunoprecipitation of flag-hBAFF by

0.5ml conditioned media from untransfected 293EBNA, (7) immunoprecipitation of flag-hTWEAK by 0.5ml conditioned media from untransfected 293EBNA.

DETAILED DESCRIPTION

I. Definitions

The term "BAFF-R" when used herein encompass native sequence BAFF-R and BAFF-R variants (which are further defined herein). The BAFF-R may be isolated from a variety of sources, such as from murine or human tissue types or from another source, or prepared by recombinant or synthetic methods.

A "native sequence BAFF-R" comprises a polypeptide having the same amino acid sequence as BAFF-R derived from nature. Such native sequence BAFF-R can be isolated from nature or can be produce by recombinant or synthetic means. The naturally-occurring truncated or secreted forms of the BAFF-R (e.g. soluble forms containing for instance, an extracellular domain sequence), naturally-occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants of the BAFF-R. In one embodiment of the invention, the native sequence BAFF-R is a mature or full-length native sequence BAFF-R polypeptide comprising amino acids 1 to 184 of SEQ ID NO: 1 or fragment thereof.

The "BAFF-R extracellular domain" or "BAFF-R ECD" refers to a form of BAFF-R which is essentially free of transmembrane and cytoplasmic domains of BAFF-R. Ordinarily, BAFF-R extracellular domain will have less than 1% of such transmembrane and cytoplasmic domains and will preferably have less than 0.5% of such domains. Optionally, BAFF-R ECD will comprise amino acid residues 8 to 41 of SEQ ID NO:1. It will be understood by the skilled artisan that the transmembrane domain identified for the BAFF-R polypeptide of the present invention is identified pursuant to criteria routinely employed in the art for identifying that type of hydrophobic domain. The exact boundaries of a transmembrane domain may vary but most likely by no more than about 5 amino acids at either end of the domain specifically mentioned herein. Accordingly, the BAFF-R ECD may optionally comprise amino acids 8-41 (SEQ ID NO:1).

"BAFF-R variant" means an active BAFF-R as defined below having at least about 80% amino acid sequence identity with the BAFF-R having the deduced amino acid

sequence shown in SEQ ID NO:1 for a full-length native sequence BAFF-R or with a BAFF-R ECD sequence. Such BAFF-R variants include, for instance, BAFF-R polypeptides wherein one or more amino acid residues are added, or deleted, at the end or C-terminus of the sequence of SEQ ID NO:1. Ordinarily, a BAFF-R variant will have at least about 80% or 85% amino acid sequence identity, more preferably at least about 90% amino acid sequence identity, and even more preferably at least about 95% amino acid sequence identity with the amino acid sequence of SEQ ID NO:1.

"Percent (%) amino acid sequence identity" with respect BAFF-R sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the BAFF-R sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publically available computer software such as BLAST, ALIGN, or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximum alignment over the full length of the sequences being compared.

The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising BAFF-R, or a domain sequence thereof, fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, or which can be identified by some other agent, yet is short enough such that it does not interfere with activity of the BAFF-R. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross - react with other epitopes. Suitable tag polypeptides generally have at least 6 amino acid residues and usually between about 8 to about 50 amino acid residues (preferably, about 10 to about 20 residues).

"Isolated" when used to describe the various polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Contaminate components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and

may include enzymes, hormones, and other proteinaceous or non- proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by us of a spinning cup sequenator, or (2) to homogeneity SDSPAGE under non-reducing or reducing conditions using Coomassie blue or preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the BAFF-R's natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

The term "antibody" is used in the broadest sense and specifically covers single BAFF-R monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies) and anti- BAFF-R antibody compositions with polyepitopic specificity. The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e. the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

A "purified preparation" or a "substantially pure preparation" of a polypeptide, as used herein, means a polypeptide that has been separated from other proteins, lipids, and nucleic acids with which it naturally occurs. Preferably, the polypeptide is also separated from other substances, e.g., antibodies, matrices, etc., which are used to purify it.

The terms, "treating", "treatment" and "therapy" as used herein refers to curative therapy, prophylactic therapy, and preventative therapy.

The terms "peptides", "proteins", and "polypeptides" are used interchangeably herein.

"Biologically active" as used herein, means having an in vivo or in vitro activity which may be performed directly or indirectly. Biologically active fragments of BAFF-R may have, for example, 70% amino acid homology with the active site of the receptor, more preferably at least 80%, and most preferably, at least 90% homology. Identity or homology with respect to the receptor is defined herein as the percentage of amino acid residues in the candidate sequence which are identical to the BAFF-R residues in SEQ. ID. NO. 1.

The term "mammal" as used herein refers to any animal classified as a mammal including humans, cows, horses, dogs, mice and cats. In preferred embodiment of the invention, the mammal is a human.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are described in the literature.

Reference will now be made in detail to the present preferred embodiments of the invention. This invention relates to the use of BAFF-R and BAFF-R related molecules to effect the growth and maturation of B-cells and the secretion of immunoglobulin. The invention relates to the use of BAFF-R and BAFF-R related molecules to effect responses of the immune system, as necessitated by immune-related disorders. Additionally, this invention encompasses the treatment of cancer and immune disorders through the use of a BAFF-R, or BAFF-R related gene through gene therapy methods.

The BAFF-R and homologs thereof produced by hosts transformed with the sequences of the invention, as well as native BAFF-R purified by the processes known in the art, or produced from known amino acid sequences, are useful in a variety of methods for anticancer, antitumor and immunoregulatory applications. They are also useful in therapy and methods directed to other diseases.

Another aspect of the invention relates to the use of the polypeptide encoded by the isolated nucleic acid encoding the BAFF-R in "antisense" therapy. As used herein, "antisense" therapy refers to administration or *in situ* generation of oligonucleotides or their derivatives which specifically hybridize under cellular conditions with the cellular mRNA and/or DNA encoding the ligand of interest, so as to inhibit expression of the encoded protein, i.e. by inhibiting transcription and/or translation. The binding may be by conventional base pair complementarity, or, for example, in the case of binding to DNA duplexes, through specific interactions in the major groove of the double helix. In general, "antisense" therapy refers to a range of techniques generally employed in the art, and includes any therapy which relies on specific binding to oligonucleotide sequences.

An antisense construct of the present invention can be delivered, for example, as an expression plasmid, which, when transcribed in the cell, produces RNA which is

complementary to at least a portion of the cellular mRNA which encodes Kay-ligand. Alternatively, the antisense construct can be an oligonucleotide probe which is generated ex vivo. Such oligonucleotide probes are preferably modified oligonucleotides which are resistant to endogenous nucleases, and are therefor stable in vivo. Exemplary nucleic acids molecules for use as antisense oligonucleotides are phosphoramidates, phosphothioate and methylphosphonate analogs of DNA (See, e.g., 5,176,996; 5,264,564; and 5,256,775). Additionally, general approaches to constructing oligomers useful in antisense therapy have been reviewed, for example, by Van Der Krol et al., (1988) Biotechniques 6:958-976; and Stein et al. (1988) Cancer Res 48: 2659-2668, specifically incorporated herein by reference.

The BAFF-R of the invention, as discussed above, is a member of the TNF receptor family. The protein, fragments or homologs thereof may have wide therapeutic and diagnostic applications.

The polypeptides of the invention specifically interact with BAFF, a polypeptide previously described in WO99/12964 incorporated by reference herein. However, the peptides and methods disclosed herein enable the identification of molecules which specifically interact with the BAFF-R or fragments thereof.

The claimed invention in certain embodiments includes methods of using peptides derived from BAFF-R which have the ability to bind to BAFF. Fragments of the BAFF-Rs can be produced in several ways, e.g., recombinantly, by PCR, proteolytic digestion or by chemical synthesis. Internal or terminal fragments of a polypeptide can be generated by removing one or more nucleotides from one end or both ends of a nucleic acid which encodes the polypeptide. Expression of the mutagenized DNA produces polypeptide fragments.

Polypeptide fragments can also be chemically synthesized using techniques known in the art such as conventional Merrifield solid phase f- moc or t-boc chemistry. For example, peptides and DNA sequences of the present invention may be arbitrarily divided into fragments of desired length with no overlap of the fragment, or divided into overlapping fragments of a desired length. Methods such as these are described in more detail below.

Generation of Soluble Forms of BAFF-R

Soluble forms of the BAFF-R can often signal effectively and hence can be administered as a drug which now mimics the natural membrane form. It is possible that the BAFF-R claimed herein are naturally secreted as soluble cytokines, however, if not, one can reengineer the gene to force secretion. To create a soluble secreted form of BAFF-R, one would remove at the DNA level the N-terminus transmembrane regions, and some portion of the stalk region, and replace them with a type I leader or alternatively a type II leader sequence that will allow efficient proteolytic cleavage in the chosen expression system. A skilled artisan could vary the amount of the stalk region retained in the secretion expression construct to optimize both ligand binding properties and secretion efficiency. For example, the constructs containing all possible stalk lengths, i.e. N-terminal truncations, could be prepared such that proteins starting at amino acids 1 to 52 would result. The optimal length stalk sequence would result from this type of analysis.

Generation of Antibodies Reactive with the BAFF-R

The invention also includes antibodies specifically reactive with the claimed BAFF-R or its co-receptors. Anti-protein/anti-peptide antisera or monoclonal antibodies can be made by standard protocols (See, for example, *Antibodies: A Laboratory Manual* ed. by Harlow and Lane (Cold Spring Harbor Press: 1988)). A mammal such as a mouse, a hamster or rabbit can be immunized with an immunogenic form of the peptide. Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers, or other techniques, well known in the art.

An immunogenic portion of BAFF-R or its co-receptors can be administered in the presence of an adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of antibodies.

In a preferred embodiment, the subject antibodies are immunospecific for antigenic determinants of BAFF-R or its co-receptors, e.g. antigenic determinants of a polypeptide of SEQ. ID. NO:1, or a closely related human or non-human mammalian homolog (e.g. 70, 80 or 90 percent homologous, more preferably at least 95 percent homologous). In yet a further preferred embodiment of the present invention, the anti-BAFF-R or anti-BAFF-co-receptor antibodies do not substantially cross react (i.e. react specifically) with a protein which is e.g., less than 80 percent homologous to SEQ. ID. NO:1; preferably less than 90

percent homologous with SEQ. ID. NO: 1; and, most preferably less than 95 percent homologous with SEQ. ID. NO:1. By "not substantially cross react", it is meant that the antibody has a binding affinity for a non-homologous protein which is less than 10 percent, more preferably less than 5 percent, and even more preferably less than 1 percent, of the binding affinity for a protein of SEQ. ID. NO. 1.

The term antibody as used herein is intended to include fragments thereof which are also specifically reactive with BAFF-R, or its receptors. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, $F(ab')_2$ fragments can be generated by treating antibody with pepsin. The resulting $F(ab')_2$ fragment can be treated to reduce disulfide bridges to produce Fab' fragments. The antibodies of the present invention are further intended to include biospecific and chimeric molecules having anti-BAFF-R or anti-BAFF-co-receptor activity. Thus, both monoclonal and polyclonal antibodies (Ab) directed against BAFF-R, and their co-receptors, and antibody fragments such as Fab` and $F(ab`)_2$, can be used to block the action of the BAFF-R and its respective co-receptors.

Various forms of antibodies can also be made using standard recombinant DNA techniques. (Winter and Milstein, Nature 349: 293-299 (1991) specifically incorporated by reference herein.) For example, chimeric antibodies can be constructed in which the antigen binding domain from an animal antibody is linked to a human constant domain (e.g. Cabilly et al., U.S: 4,816,567, incorporated herein by reference). Chimeric antibodies may reduce the observed immunogenic responses elicited by animal antibodies when used in human clinical treatments.

In addition, recombinant "humanized antibodies" which recognize BAFF-R or its co-receptors can be synthesized. Humanized antibodies are chimeras comprising mostly human IgG sequences into which the regions responsible for specific antigen-binding have been inserted. Animals are immunized with the desired antigen, the corresponding antibodies are isolated, and the portion of the variable region sequences responsible for specific antigen binding are removed. The animal-derived antigen binding regions are then cloned into the appropriate position of human antibody genes in which the antigen binding regions have been deleted. Humanized antibodies minimize the use of heterologous (i.e.

inter species) sequences in human antibodies, and thus are less likely to elicit immune responses in the treated subject.

Construction of different classes of recombinant antibodies can also be accomplished by making chimeric or humanized antibodies comprising variable domains and human constant domains (CH1, CH2, CH3) isolated from different classes of immunoglobulins. For example, antibodies with increased antigen binding site valencies can be recombinantly produced by cloning the antigen binding site into vectors carrying the human : chain constant regions. (Arulanandam et al., J. Exp. Med., 177: 1439-1450 (1993), incorporated herein by reference.)

In addition, standard recombinant DNA techniques can be used to alter the binding affinities of recombinant antibodies with their antigens by altering amino acid residues in the vicinity of the antigen binding sites. The antigen binding affinity of a humanized antibody can be increased by mutagenesis based on molecular modeling. (Queen et al., Proc. Natl. Acad. Sci. 86: 10029-33 (1989) incorporated herein by reference.

Generation of Analogs: Production of Altered DNA and Peptide Sequences

Analogs of the BAFF-R can differ from the naturally occurring BAFF-R in amino acid sequence, or in ways that do not involve sequence, or both. Non-sequence modifications include in vivo or in vitro chemical derivatization of the BAFF-R. Non-sequence modifications include, but are not limited to, changes in acetylation, methylation, phosphorylation, carboxylation or glycosylation.

Preferred analogs include BAFF-R biologically active fragments thereof, whose sequences differ from the sequence given in SEQ. ID NO. 1, by one or more conservative amino acid substitutions, or by one or more non-conservative amino acid substitutions, deletions or insertions which do not abolish the activity of BAFF-ligand. Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics, e.g. substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and, phenylalanine, tyrosine.

Uses

The full length BAFF-R gene (SEQ ID NO 2) or portions thereof may be used as hybridization probes for a cDNA library to isolate, for instance, still other genes which

have a desired sequence identity to the BAFF-R sequence disclosed in SEQ ID NO. 2. Nucleotide sequences encoding BAFF-R can also be used to construct hybridization probes for mapping the gene which encodes the BAFF-R and for the genetic analysis of individuals with genetic disorders. Screening assays can be designed to find lead compounds that mimic the biological activity of a BAFF-R. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules contemplated include synthetic organic or inorganic compounds. Nucleic acids which encode BAFF-R or its modified forms can also be used to generate either transgenic animals or "knock out" animals which in turn are useful in the development and screening of therapeutically useful reagents.

As described herein, in one embodiment of the invention, there are provided methods of stimulating B-cell growth, dendritic cell-induced B-cell growth and maturation or immunoglobulin production in an animal using BAFF-R polypeptide or co-stimulating B-cell growth, dendritic cell-induced B-cell growth and maturation or immunoglobulin production in an animal using BAFF-R polypeptide and an anti-T antibody, a CD40 ligand or an anti-CD40 ligand. Also included are methods of inhibiting B-cell growth, dendritic cell-induced B-cell growth and maturation or immunoglobulin production in an animal using BAFF-R polypeptide.

In another embodiment, the invention provides methods of using BAFF-R in the treatment of autoimmune diseases, hypertension, cardiovascular disorders, renal disorders, B-cell lympho-proliferate disorders, immunosuppressive diseases, organ transplantation, inflammation, and HIV. Also included are methods of using agents for treating, suppressing or altering an immune response involving a signaling pathway between BAFF-R and its ligand.

In one embodiment, the invention provides pharmaceutical compositions comprising a BAFF-R polypeptide and a pharmaceutically acceptable excipient. Suitable carriers for a BAFF-R polypeptide, for instance, and their formulations, are described in Remington' Pharmaceutical Sciences, 16th ed., 1980, Mack Publishing Co., edited by Oslo et al. Typically an appropriate amount of a pharmaceutically acceptable salt is used in the formulation to render the formulation isotonic. Examples of the carrier include buffers

such as saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7.4 to about 7.8. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers, which matrices are in the form of shaped articles, e.g. liposomes, films or microparticles. It will be apparent to those of skill in the art that certain carriers may be more preferable depending upon for instance the route of administration and concentration of the a BAFF-R polypeptide being administered.

Administration may be accomplished by injection (eg intravenous, intraperitoneal, subcutaneous, intramuscular) or by other methods such as infusion that ensure delivery to the bloodstream in an effective form.

Practice of the present invention will employ, unless indicated otherwise, conventional techniques of cell biology, cell culture, molecular biology, microbiology, recombinant DNA, protein chemistry, and immunology, which are within the skill of the art. Such techniques are described in the literature. See, for example, **Molecular Cloning: A Laboratory Manual**, 2nd edition. (Sambrook, Fritsch and Maniatis, eds.), Cold Spring Harbor Laboratory Press, 1989; **DNA Cloning**, Volumes I and II (D.N. Glover, ed), 1985; **Oligonucleotide Synthesis**, (M.J. Gait, ed.), 1984; U.S. Patent No. 4,683,195 (Mullis et al.,); **Nucleic Acid Hybridization** (B.D. Hames and S.J. Higgins, eds.), 1984; **Transcription and Translation** (B.D. Hames and S.J. Higgins, eds.), 1984; **Culture of Animal Cells** (R.I. Freshney, ed). Alan R. Liss, Inc., 1987; **Immobilized Cells and Enzymes**, IRL Press, 1986; **A Practical Guide to Molecular Cloning** (B. Perbal), 1984; **Methods in Enzymology**, Volumes 154 and 155 (Wu et al., eds), Academic Press, New York; **Gene Transfer Vectors for Mammalian Cells** (J.H. Miller and M.P. Calos, eds.), 1987, Cold Spring Harbor Laboratory; **Immunochemical Methods in Cell and Molecular Biology** (Mayer and Walker, eds.), Academic Press, London, 1987; **Handbook of Experiment Immunology**, Volumes I-IV (D.M. Weir and C.C. Blackwell, eds.), 1986; **Manipulating the Mouse Embryo**, Cold Spring Harbor Laboratory Press, 1986.

The following Examples are provided to illustrate the present invention, and should not be construed as limiting thereof.

EXAMPLES:

Example 1: Detection of BAFF binding to BAFF-R using a Plate Assay

In this example, the binding of BAFF to BAFF-R transfected cells using a plate assay, is described.

Full-length human BAFF-R was generated from BJAB polyA+ RNA using the SuperscriptII preamplification kit (Life Technologies) to generate the cDNA template and amplified by Pfu1 using primers complementary to the 5' and 3' coding sequences of BAFF-R. The PCR product was cloned into CH269, a derivative of pCEP4 (Invitrogen). The resultant clone was termed pJST535. Human embryonic kidney cells containing the EBNA-1 gene (293EBNA) were seeded into 6 well plates coated with fibronectin and transfected by lipofectamine (Life Technologies) with either pJST535 at various dilutions or CH269 as a background control. At 48 hrs post transfection, transfected cells were assayed for their ability to bind soluble flag-hBAFF(amino acids L83-L285) as follows. All incubations were at room temperature. Conditioned media was aspirated from the wells and the cells washed with BHA buffer (20mM HEPES pH7.0, 0.5mg/ml BSA, 0.1% NaN3) and incubated with 0.5ug/ml FLAG-hBAFF diluted in PBS containing 1mM MgCl2, 1mM CaCl2 and 0.1% NaN3. After an 1 hr. incubation, the BAFF solution was removed and the cells were washed with BHA. The cells were next incubated for 30min. in a PBS solution containing 1ug/ml of the anti-FLAG monoclonal antibody, M2 (Sigma). This solution was aspirated and the cells were washed with BHA. The cells were then incubated for 30 min. in a 1:3000 dilution of the alkaline phosphatase conjugated goat anti-mouse IgG F(ab)'2 (Jackson ImmunoResearch). This solution was aspirated and the cells washed with BHA. To reduce the amount of background staining due to endogenous alkaline phosphatase, the cells were incubated for 15 min. in 2.5mM levamisol (Vector Laboratories) diluted in 100mM NaCl, 100mM Tris-Cl pH9.5 and 5mM MgCl2. For chromogenic detection of alkaline phosphatase, the inhibitor solution was aspirated and the cells were incubated with a solution of fast red and napthol phosphate (Pierce). Staining was observed and photographed through a low power microscope.

Results:

The deposition of fast red dye was observed for all the wells transfected with the BAFF-R expressing plasmid, pJST535. The frequency of the signal titrated away as the amount of plasmid transfected into the cells decreased. No staining was observed for the control expression vector, CH269, transfected cells. Also, no staining was observed on any of the transfected cells when FLAG-hBAFF was omitted from the staining staining protocol or when another TNF family member ligand, FLAG-tagged LIGHT, was substituted for flag-hBAFF. Therefore the staining of BAFF-R transfected cells with BAFF appears specific.

Example 2: BAFF Binding to BAFF-R transfected cells by FACS analysis

This example describes the detection of BAFF to BAFF-R transfected cells using FACS analysis.

The plasmid encoding full-length BAFF-R, pJST535, was transfected into 293EBNA cells using FuGene6 (Boehringer Mannheim). At 24 or 48hr post transfection, cells were removed from the plates using 5mM EDTA in PBS and counted. The cells were washed twice with FACS buffer (PBS containing 10% fetal bovine serum, 0.1% NaN3 and 10 ug/ml hIgG (Jackson ImmunoResearch) and then 2.5 x 10^5 cells were incubated for 1hr on ice with FLAG-hBAFF diluted into FACS buffer at concentrations ranging from 9 ug/ml to 0.037 ug/ml. The cells were washed with FACS buffer and incubated with the anti-FLAG monoclonal antibody, M2, at 5 ug/ml for 30 min. on ice. The cells were washed with FACS buffer and incubated for 30 min. on ice in a solution containing a 1:100 dilution of R-phycoerythrin conjugated F(ab')2 donkey anti-mouse IgG and 10ug/ml 7-AAD. After the cells were washed with FACS buffer, they were resuspended in FACS buffer containing 1% paraformaldehyde. FACS anaysis followed where the 7-AAD positive (dead) cells were gated out.

Results:

The results of the FACS analysis indicate that a fraction of the cells that were transfected with BAFF-R are able to bind BAFF. At a BAFF concentration of 9 ug/ml, about 28% of the cells bind BAFF for a mean fluorescence intensity (MFI) of 366. Using the PE-labeled donkey anti-mouse reagent alone, there is not a significant shift of the cells. Only 1.3% of the cells have a MFI of 23.5 with the average of all the cells being 5.5. The signal on the

BAFF-R transfected cells titrates out with decreasing amounts of BAFF. At 100 ng/ml, 8.76% of the cells have an MFI of 78.9.

Example 3: FACS analysis of BAFF/BAFF-R interaction including a GFP marker

In this example, the ability of BAFF to bind to cells co-transfected with BAFF-R and a GFP reporter plasmid is described.

The 293EBNA cells were transfected with pJST535 and a GFP reporter plasmid, as described in example 2. The reporter plasmid encodes a membrane anchored GFP molecule. Using co-transfection of the two plasmids, we analyzed the percentage of transfected cells which were capable of binding BAFF. The cells were removed from the plates and subjected to BAFF binding and detection as described in example 2. Again, 7-AAD was included in order to gate out the dead cells. The samples were analyzed by FACS and plotted. The upper right quadrant represents cells with bound BAFF (phycoerythrin positive) and expressing GFP.

Results:

Although not all of the GFP transfected cells appear to bind BAFF, there is a significant fraction of the cells in the upper right quadrant compared to the control. Thirty-three percent of the transfected cells are in the upper right compared to 8 percent It may be that a certain level of BAFF-R expression is required for BAFF to bind to the cells. It is also possible that a co-receptor is required for a high affinity interaction and that this receptor is limiting on the 293EBNA cells.

Example 4: Immunoprecipitation of flag-hBAFF by BAFF-R-Fc Fusion

This example describes specific interaction of flag-hBAFF with BAFF-R-Fc, a molecule composed of the Cysteine Rich Domain (CRD) of BAFF-R fused to the Fc domain of human IgG1.

The CRD of BAFF-R was generated by RT-PCR from BJAB polyA+ RNA as in Example 1 using as a 3' primer an oligo complementary to nucleotides 132-152 of the hBAFF-R coding sequence. The resultant PCR fragment was cloned in CH269

downstream of a murine IgG-kappa signal sequence and upstream of the Fc moiety of human IgG. This construct was termed pJST538. The construct pJST538 or CH269 were transfected to 293EBNA by lipofectamine. Conditioned media and cell extracts were harvested at 20 hours post transfection. Cells were solublized in 20mM Tris pH7.5/50mM Nacl/ 0.5% NP40/ 0.5% deoxycholic acid and debris spun out. An aliquot of the conditioned media and cell extracts were combined with an equal volume 2x SDS reducing buffer, boiled and subjected to SDS-PAGE and Western transfer. To verify expression, the membrane was probed with 1:3000 dilution of mouse anti-human IgG conjugated to horse radish peroxidase (HRP) in 5% nonfat dry-milk in TBST at room temperature for 30 minutes and washed with TBST. Blots were developed with ECL (Amersham) and exposed to film.

Immunoprecipitations were performed by incubating 25ng of either flag-hBAFF or flag-hTWEAK with 0.5 ml of conditioned media from 293EBNA transfected with either pJST538 or CH269 at 4*C for 1 hour with agitation followed by the addition of 30ul ProteinA-Sepharose (Pharmacia) and continued agitation overnight. ProteinA-Sepharose beads were washed twice with PBS and resuspended in 2xSDS reducing sample buffer. After SDS-PAGE and western transfer, the blots were incubated with 5ug/ml anti-flag monoclonal antibody M2 (Sigma) in 5% nonfat dry milk in TBST at room temperature for 1 hour. Blots washed with TBST and incubated with a 1:3000 dilution of goat anti-mouse IgG HRP conjugate (Jackson Immunoresearch) in 5% nonfat dry milk in TBST at room temperature for 30 minutes. Blots were developed with ECL (Amersham) and exposed to film.

Results:

Upon transfection of pJST538 to 293EBNA cells, expression of an approximately 43kDa protein was detected in both the cell extract and conditioned medium by Western blot analysis with mouse anti-human IgG (Jackson Immunoresearch), indicating that the BAFF-R-Fc fusion was efficiently expressed and secreted.

In the immunoprecipitations a band was observed only as a result of the incubation of BAFF-R-Fc and flag-hBAFF. This band co-migrated with a directly loaded sample of flag-hBAFF. None of the other lanes produced a signal, indicating that the interaction between BAFF-R-Fc and flag-hBAFF is specific.

Example 5: Generation of Soluble Receptor Forms:

To form an receptor inhibitor for use in man, one requires the human receptor cDNA sequence of the extracellular domain. If the mouse form is known, human cDNA libraries can be easily screened using the mouse cDNA sequence and such manipulations are routinely carried out in this area. With a human cDNA sequence, one can design oligonucleotide primers to PCR amplify the extracellular domain of the receptor in the absence of the transmembrane and intracellular domains. Typically, one includes most of the amino acids between the last disulfide linked "TNF domain" and the transmembrane domain. One could vary the amount of "stalk" region included to optimize the potency of the resultant soluble receptor. This amplified piece would be engineered to include suitable restriction sites to allow cloning into various C-terminal Ig fusion chimera vectors. Alternatively, one could insert a stop signal at the 3' end and make a soluble form of the receptor without resorting to the use of a Ig fusion chimera approach. The resultant vectors can be expressed in most systems used in biotechnology including yeast, insect cells, bacteria and mammalian cells and examples exist for all types of expression. Various human Fc domains can be attached to optimize or eliminate FcR and complement interactions as desired. Alternatively, mutated forms of these Fc domains can be used to selectively remove FcR or complement interactions or the attachment of N-linked sugars to the Fc domain which has certain advantages.

Example 6: Generation of Agonistic or Antagonistic Antibodies:

The above described soluble receptor forms can be used to immunize mice and to make monoclonal antibodies by conventional methods. The resultant mAbs that are identified by ELISA methods can be further screened for agonist activity either as soluble

antibodies or immobilized on plastic in various in vitro cellular assays. Often the death of the HT29 cell line is a convenient system that is sensitive to signaling through many TNF receptors. If this line does not possess the receptor of interest, that full length receptor can be stably transfected into the HT29 line to now allow the cytotoxicity assay to work. Alternatively, such cells can be used in the Cytosensor apparatus to assess whether activation of the receptor can elicit a pH change that is indicative of a signaling event. TNF family receptors signal well in such a format and this method does not require one to know the actual biological events triggered by the receptor. The agonistic mAbs would be "humanized" for clinical use. This procedure can also be used to define antagonistic mAbs. Such mAbs would be defined by the lack of agonist activity and the ability to inhibit receptor-ligand interactions as monitored by ELISA, classical binding or BIAcore techniques. Lastly, the induction of chemokine secretion by various cells in response to an agonist antibody can form a screening assay.

Example 7: Screening for Inhibitors of the Receptor-Ligand Interaction:

Using the receptor-Ig fusion protein, one can screen either combinatorial libraries for molecules that can bind the receptor directly. These molecules can then be tested in an ELISA formatted assay using the receptor-Ig fusion protein and a soluble form of the ligand for the ability to inhibit the receptor-ligand interaction. This ELISA can be used directly to screen various natural product libraries etc. for inhibitory compounds. The receptor can be transfected into a cell line such as the HT29 line to form a biological assay (in this case cytotoxicity) that can then form the screening assay.

It will be apparent to those skilled in the art that various modifications and variations can be made in the polypeptides, compositions and methods of the invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided that they come within the scope of the appended claims and their equivalents.

What is claimed is:

1. A method of stimulating B-cell growth in an animal comprising the step of administering a therapeutically effective amount of a composition comprising a BAFF-R or an active fragment thereof.

2. The method according to claim 1, wherein the BAFF-R is a soluble BAFF-R.

3. The method according to claim 2, wherein the soluble BAFF-R is a recombinant BAFF-R.

4. The method according to claim 1, wherein the animal is of mammalian origin.

5. The method according to claim 4, wherein the mammal is human.

6. A method of stimulating immunoglobulin production in an animal comprising the step of administering a therapeutically effective amount of a composition comprising a BAFF-R or an active fragment thereof.

7. The method according to claim 6, wherein the BAFF-R is a soluble BAFF-R.

8. The method according to claim 7, wherein the soluble BAFF-R is a recombinant BAFF-R.

9. The method according to claim 6, wherein the animal is of mammalian origin.

10. The method according to claim 9, wherein the mammal is human.

11. A method of co-stimulating B-cell growth and immunoglobulin production in an animal comprising the step of administering a therapeutically effective amount of a composition comprising a BAFF-R or an active fragment thereof.

12. The method according to claim 11, wherein the BAFF-R is a soluble BAFF-R.

13. The method according to claim 12, wherein the soluble BAFF-R is a recombinant BAFF-R.

14. The method according to claim 11, wherein the animal is of mammalian origin.

15. The method according to claim 14, wherein the mammal is human.

16. A method of stimulating dendritic cell-induced B-cell growth and maturation comprising the step of administering a therapeutically effective amount of a composition comprising a BAFF-R or an active fragment thereof.

17. The method according to claim 16, wherein the BAFF-R is a soluble BAFF-R.

18. The method according to claim 17, wherein the soluble BAFF-R is a recombinant BAFF-R.

19. The method according to claim 16, wherein the animal is of mammalian origin.

20. The method according to claim 19, wherein the mammal is human.

21. A method of inhibiting B-cell growth in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of:

(a) a anti-BAFF-R molecule or an active fragment thereof;
(b) a recombinant, inoperative BAFF-R molecule or an active fragment thereof;
(c) an antibody specific for BAFF-R or an active fragment thereof; and
(d) an antibody specific for BAFF-R or an epitope thereof.

22. The method according to claim 21, wherein the anti-BAFF-R is soluble.

23. The method according to claim 22, wherein the soluble anti-BAFF-R is a recombinant anti-BAFF-R.

24. The method according to claim 21, wherein the anti-BAFF antibody is a monoclonal antibody.

25. The method according to claim 21, wherein the animal is of mammalian origin.

26. The method according to claim 25, wherein the mammal is human.

27. A method of inhibiting immunoglobulin production in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of:

(a) a anti-BAFF-R molecule or an active fragment thereof;
(b) a recombinant, inoperative BAFF-R molecule or an active fragment thereof;
(c) an antibody specific for BAFF-R or an active fragment thereof; and
(d) an antibody specific for BAFF-R or an epitope thereof.

28. The method according to claim 27, wherein the anti-BAFF-R is soluble.

29. The method according to claim 28, wherein the soluble anti-BAFF-R is a recombinant anti-BAFF-R.

30. The method according to claim 27, wherein the anti-BAFF-R antibody is a monoclonal antibody.

31. The method according to claim 27, wherein the animal is of mammalian origin.

32. The method according to claim 31, wherein the mammal is human.

33. A method of co-inhibiting B-cell growth and immunoglobulin production in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of:
(a) a anti-BAFF-R molecule or an active fragment thereof;
(b) a recombinant, inoperative BAFF-R molecule or an active fragment thereof;
(c) an antibody specific for BAFF-R or an active fragment thereof; and
(d) an antibody specific for BAFF-R or an epitope thereof.

34. The method according to claim 33, wherein the anti-BAFF-R is soluble.

35. The method according to claim 34, wherein the soluble anti-BAFF-R is a recombinant anti-BAFF-R.

36. The method according to claim 33, wherein the anti-BAFF-R antibody is a monoclonal antibody.

37. The method according to claim 33, wherein the animal is of mammalian origin.

38. The method according to claim 37, wherein the mammal is human.

39. A method of inhibiting dendritic cell-induced B-cell growth and maturation in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of:
(a) a anti-BAFF-R molecule or an active fragment thereof;
(b) a recombinant, inoperative BAFF-R molecule or an active fragment thereof;
(c) an antibody specific for BAFF ligand or an active fragment thereof; and
(d) an antibody specific for BAFF-R or an epitope thereof.

40. The method according to claim 39, wherein the anti-BAFF-R is soluble.

41. The method according to claim 40, wherein the soluble anti-BAFF-R is a recombinant anti-BAFF-R.

42. The method according to claim 39, wherein the anti-BAFF-R antibody is a monoclonal antibody.

43. The method according to claim 39, wherein the animal is of mammalian origin.

44. The method according to claim 43, wherein the mammal is human.

45. A method of treatment of an autoimmune disease comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of:
(a) a BAFF-R or an active fragment thereof;
(b) a anti-BAFF-R molecule or an active fragment thereof;
(c) a recombinant, inoperative BAFF-R molecule or an active fragment thereof;
(d) an antibody specific for BAFF-R or an active fragment thereof; and
(e) an antibody specific for BAFF-R or an epitope thereof.

46. The method according to claim 45, wherein the BAFF-R is a soluble BAFF-R.

47. The method according to claim 46, wherein the soluble BAFF-R is a recombinant BAFF-R.

48. The method according to claim 45, wherein the anti-BAFF-R is soluble.

49. The method according to claim 48, wherein the soluble anti-BAFF-R is a recombinant anti-BAFF-R.

50. The method according to claim 45, wherein the anti-BAFF-R antibody is a monoclonal antibody.

51. The method according to claim 45, wherein the animal is of mammalian origin.

52. The method according to claim 45, wherein the mammal is human.

53. A method of treating a disorder related to BAFF-ligand comprising the steps of:
(a) introducing into a desired cell a therapeutically effective amount of a vector containing a gene encoding for a BAFF-related molecule; and
(b) expressing said gene in said cell.

54. The method according to claim 53, wherein the BAFF-related molecule is selected from the group consisting of:
(a) a BAFF-R or an active fragment thereof;
(b) a anti-BAFF-R molecule or an active fragment thereof;
(c) a recombinant, inoperative BAFF-R molecule or an active fragment thereof;
(d) an antibody specific for BAFF-R or an active fragment thereof; and
(e) an antibody specific for BAFF-R or an epitope thereof.

55. The method according to claim 54, wherein the BAFF-R is a soluble BAFF-R.

56. The method according to claim 55, wherein the soluble BAFF-R is a recombinant BAFF-R.

57. The method according to claim 54, wherein the anti-BAFF-R is soluble.

58. The method according to claim 54, wherein the soluble anti-BAFF-R is a recombinant anti-BAFF-R.

59. The method according to claim 54, wherein the anti-BAFF-R antibody is a monoclonal antibody.

60. The method according to claim 54, wherein the animal is of mammalian origin.

61. The method according to claim 60, wherein the mammal is human.

62. A method of inducing cell death comprising the administration of an agent capable of interfering with the binding of a BAFF-R to BAFF.

63. A method of treating, suppressing or altering an immune response involving a signaling pathway between a BAFF-R and BAFF comprising the step of administering an effective amount of an agent capable of interfering with the association between the BAFF-R and BAFF.

64. A method of inhibiting inflammation comprising the step of administering a therapeutically effective amount of an antibody specific for a BAFF-R or an active fragment thereof.

65. A method of inhibiting inflammation comprising the step of administering a therapeutically effective amount of an antibody specific for a BAFF-R or an epitope thereof.

66. A method of regulating hematopoietic cell development comprising the step of administering a therapeutically effective amount of a BAFF-ligand or an active fragment thereof.

67. A method of treating, suppressing or altering an immune response involving a signaling pathway between a BAFF-R and BAFF comprising the step of administering an effective amount of an agent capable of interfering with the association BAFF-R and BAFF.

68. A method of treating hypertension in an animal comprising the step of administering a therapeutically effective amount of a B-cell growth inhibitor.

69. The method according to claim 68, wherein the B-cell growth inhibitor is selected from the group consisting of:

(a) a anti-BAFF-R molecule or an active fragment thereof;
(b) a recombinant, inoperative BAFF-R molecule or an active fragment thereof;
(c) an antibody specific for BAFF-R or an active fragment thereof; and
(d) an antibody specific for BAFF-R or an epitope thereof.

70. The method according to claim 69, wherein the anti-BAFF-R is soluble.

71. The method according to claim 70, wherein the soluble anti-BAFF-R is a recombinant anti-BAFF-R.

72. The method according to claim 69, wherein the anti-BAFF-R antibody is a monoclonal antibody.

73. The method according to claim 69, wherein the animal is of mammalian origin.

74. The method according to claim 73, wherein the mammal is human.

75. A method of treating hypertension in an animal comprising the step of administering a therapeutically effective amount of a co-inhibitor of B-cell growth and immunoglobulin secretion.

76. A method of treating cardiovascular disorders in an animal comprising the step of administering a therapeutically effective amount of a B-cell growth inhibitor.

77. A method of treating cardiovascular disorders in an animal comprising the step of administering a therapeutically effective amount of a co-inhibitor of B-cell growth and immunoglobulin production.

78. A method of treating renal disorders in an animal comprising the step of administering a therapeutically effective amount of a B-cell growth inhibitor.

79. A method of treating renal disorders in an animal comprising the step of administering a therapeutically effective amount of a co-inhibitor of B-cell growth and immunoglobulin production

80. A method of treating B-cell lympho-proliferate disorders comprising the step of administering a therapeutically effective amount of a B-cell growth inhibitor.

81. A method of stimulating B-cell production in the treatment of immunosuppressive diseases comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of:

(e) a BAFF-R or an active fragment thereof;
(f) a anti-BAFF-R molecule or an active fragment thereof;
(g) a recombinant, inoperative BAFF-R molecule or an active fragment thereof;
(h) an antibody specific for BAFF-R or an active fragment thereof; and
(i) an antibody specific for BAFF-R or an epitope thereof.

82. A method of stimulating B-cell production in the treatment of an immunosuppressive disease comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of:

(j) a BAFF-R or an active fragment thereof;

(k) a anti-BAFF-R molecule or an active fragment thereof;
(l) a recombinant, inoperative BAFF-R molecule or an active fragment thereof; and
(m)an antibody specific for BAFF-R or an active fragment thereof; and
(n) an antibody specific for BAFF-R or an epitope thereof.

83. A method according to claim 82 wherein the immunosuppressive disease is HIV.

84. A method according to claim 82 wherein the immunosuppressive disease is associated with an organ transplantation.

85. A pharmaceutical composition comprising a therapeutically effective amount of an isolated BAFF-R polypeptide or a fragment thereof and a pharmaceutically acceptable carrier.

86. The isolated BAFF-R polypeptide of claim 85 wherein the BAFF-R polypeptide is selected from the group consisting of:

a) an isolated native sequence BAFF-R polypeptide comprising amino acid residues 1 to 184 of SEQ ID NO:1 or a fragment thereof;

b) an isolated BAFF-R polypeptide having at least 80% amino acid sequence identity with native sequence BAFF-R polypeptide comprising amino acid residues 1 to 184 of SEQ ID NO: 1 or a fragment thereof;

c) an isolated BAFF-R polypeptide having at least 90% amino acid sequence identity with native sequence BAFF-R polypeptide comprising amino acid residues 1 to 184 of SEQ ID NO: 1 or a fragment thereof;

d) an isolated sequence BAFF-R polypeptide comprising amino acid residues 1 to 52 of SEQ ID NO. 1 or a fragment thereof; and

e) an isolated sequence BAFF-R polypeptide comprising amino acid residues 8 to 41 of SEQ ID NO. 1 or a fragment thereof.

87. A chimeric molecule comprising:

a) a BAFF-R polypeptide selected from the group consisting of:

i) an isolated native sequence BAFF-R polypeptide comprising amino acid residues 1 to 184 of SEQ ID NO:1 or a fragment thereof;

ii) an isolated BAFF-R polypeptide having at least 80% amino acid sequence identity with native sequence BAFF-R polypeptide comprising amino acid residues 1 to 184 of SEQ ID NO: 1 or a fragment thereof;

ii) an isolated BAFF-R polypeptide having at least 90% amino acid sequence identity with native sequence BAFF-R polypeptide comprising amino acid residues 1 to 184 of SEQ ID NO: 1 or a fragment thereof;

iii) an isolated sequence BAFF-R polypeptide comprising amino acid residues 1 to 52 of SEQ ID NO. 1 or a fragment thereof; and

iv) an isolated sequence BAFF-R polypeptide comprising amino acid residues 8 to 41 of SEQ ID NO. 1 or a fragment thereof,

b) fused to a heterologous amino acid sequence.

88. The chimeric molecule of claim 87 wherein the heterologous amino acid sequence is an immunoglobulin sequence.

89. The chimeric molecule of claim 88 wherein the immunoglobulin sequence is an IgG Fc domain.

c) An antibody which binds to a BAFF-R polypeptide selected from the group consisting of:

i) an isolated native sequence BAFF-R polypeptide comprising amino acid residues 1 to 184 of SEQ ID NO:1 or a fragment thereof;

ii) an isolated BAFF-R polypeptide having at least 80% amino acid sequence identity with native sequence BAFF-R polypeptide comprising amino acid residues 1 to 184 of SEQ ID NO: 1 or a fragment thereof;

ii) an isolated BAFF-R polypeptide having at least 90% amino acid sequence identity with native sequence BAFF-R polypeptide comprising amino acid residues 1 to 184 of SEQ ID NO: 1 or a fragment thereof;

iii) an isolated sequence BAFF-R polypeptide comprising amino acid residues 1 to 52 of SEQ ID NO. 1 or a fragment thereof; and

iv) an isolated sequence BAFF-R polypeptide comprising amino acid residues 8 to 41 of SEQ ID NO. 1 or a fragment thereof.

90. The antibody of claim 89 wherein the antibody is a monoclonal antibody.

91. The antibody of claim 89 which comprises a chimeric antibody.

92. The antibody of claim 89 which comprises a human antibody.

ABSTRACT

A novel receptor in the TNF family is provided: BAFF-R. Chimeric molecules and antibodies to BAFF-R and methods of use thereof are also provided.

FIGURE 1

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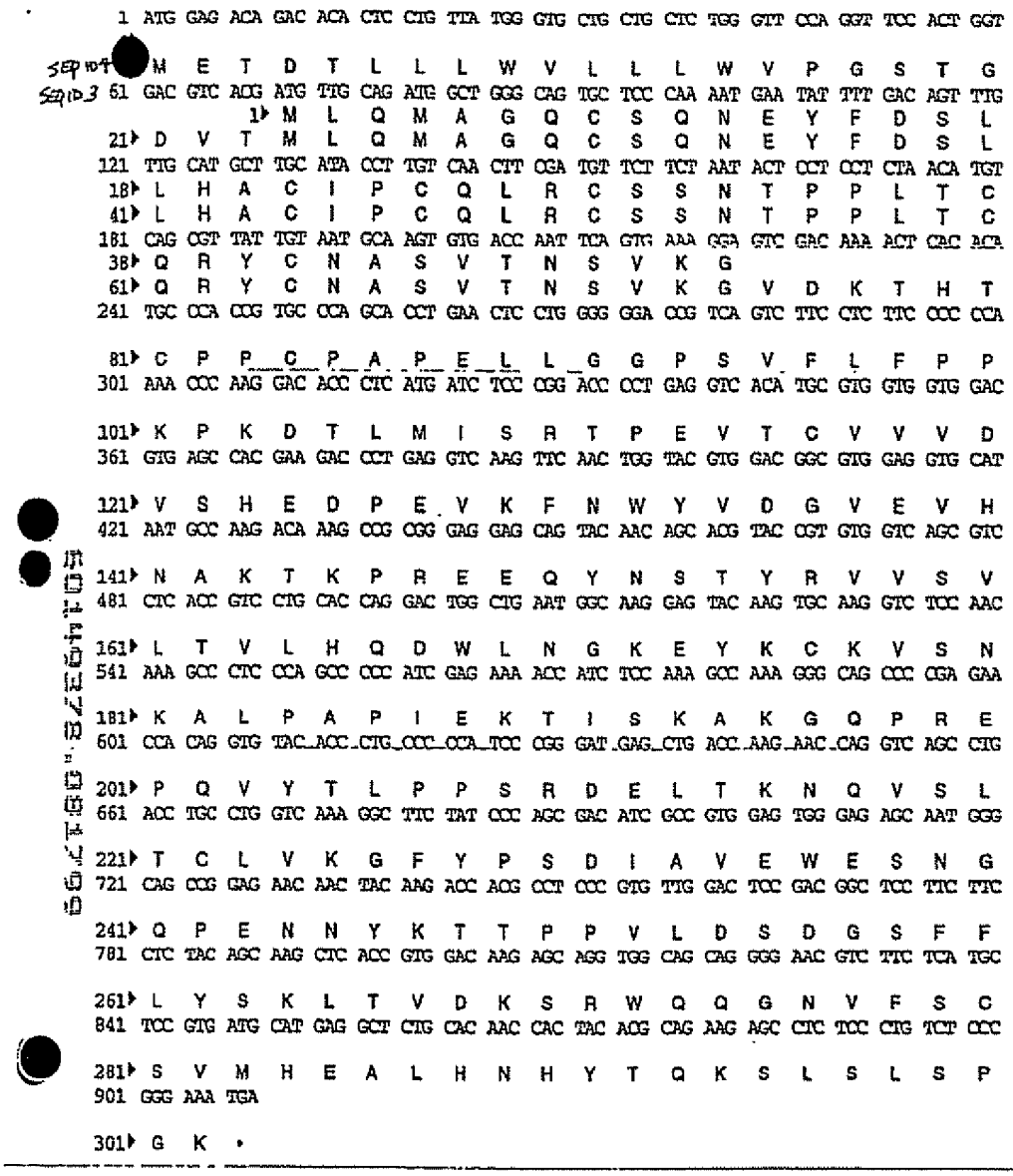
SEQ ID:2 1   ATG TTG CAG ATG GCT GGG CAG TGC TCC CAA AAT GAA TAT TTT GAC AGT TTG TTG CAT GCT
SEQ ID:1 1▶   M   L   Q   M   A   G   Q   C   S   Q   N   E   Y   F   D   S   L   L   H   A
             TGC ATA CCT TGT CAA CTT CGA TGT TCT TCT AAT ACT CCT CCT CTA ACA TGT CAG CGT TAT
     21▶      C   I   P   C   Q   L   R   C   S   S   N   T   P   P   L   T   C   Q   R   Y
     121     TGT AAT GCA AGT GTG ACC AAT TCA GTG AAA GGA ACG AAT GCG ATT CTC TGG ACC TGT TTG
     41▶      C   N   A   S   V   T   N   S   V   K   G   T   N   A   I   L   W   T   C   L
     181     GGA CTG AGC TTA ATA ATT TCT TTG GCA GTT TTC GTG CTA ATG TTT TTG CTA AGG AAG ATA
     61▶      G   L   S   L   I   I   S   L   A   V   F   V   L   M   F   L   L   R   K   I
     241     AGC TCT GAA CCA TTA AAG GAC GAG TTT AAA AAC ACA GGA TCA GGT CTC CTG GGC ATG GCT
     81▶      S   S   E   P   L   K   D   E   F   K   N   T   G   S   G   L   L   G   M   A
     301     AAC ATT GAC CTG GAA AAG AGC AGG ACT GGT GAT GAA ATT ATT CTT CCG AGA GGC CTC GAG
     101▶     N   I   D   L   E   K   S   R   T   G   D   E   I   I   L   P   R   G   L   E
     361     TAC ACG GTG GAA GAA TGC ACC TGT GAA GAC TGC ATC AAG AGC AAA CCG AAG GTC GAC TCT
     121▶     Y   T   V   E   E   C   T   C   E   D   C   I   K   S   K   P   K   V   D   S
     421     GAC CAT TGC TTT CCA CTC CCA GCT ATG GAG GAA GGC GCA ACC ATT CTT GTC ACC ACG AAA
     141▶     D   H   C   F   P   L   P   A   M   E   E   G   A   T   I   L   V   T   T   K
     481     ACG AAT GAC TAT TGC AAG AGC CTG CCA GCT GCT TTG AGT GCT ACG GAG ATA GAG AAA TCA
     161▶     T   N   D   Y   C   K   S   L   P   A   A   L   S   A   T   E   I   E   K   S
     541     ATT TCT GCT AGG TAA
     181▶     I   S   A   R   •
```

FIGURE 2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M E T D T L L L W V L L L W V P G S T G
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1 M L Q M A G Q C S Q N E Y F D S L
21 D V T M L Q M A G Q C S Q N E Y F D S L
121 TTG CAT GCT TGC ATA CCT TGT CAA CTT CGA TGT TCT TCT AAT ACT CCT CCT CTA ACA TGT
18 L H A C I P C Q L R C S S N T P P L T C
41 L H A C I P C Q L R C S S N T P P L T C
181 CAG CGT TAT TGT AAT GCA AGT GTG ACC AAT TCA GTG AAA GGA GTC GAC AAA ACT CAC ACA
38 Q R Y C N A S V T N S V K G
61 Q R Y C N A S V T N S V K G V D K T H T
241 TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA
81 C P P C P A P E L L G G P S V F L F P P
301 AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC
101 K P K D T L M I S R T P E V T C V V V D
361 GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT
121 V S H E D P E V K F N W Y V D G V E V H
421 AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC
141 N A K T K P R E E Q Y N S T Y R V V S V
481 CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC
161 L T V L H Q D W L N G K E Y K C K V S N
541 AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA
181 K A L P A P I E K T I S K A K G Q P R E
601 CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG
201 P Q V Y T L P P S R D E L T K N Q V S L
661 ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG
221 T C L V K G F Y P S D I A V E W E S N G
721 CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG TTG GAC TCC GAC GGC TCC TTC TTC
241 Q P E N N Y K T T P P V L D S D G S F F
781 CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC
261 L Y S K L T V D K S R W Q Q G N V F S C
841 TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCC
281 S V M H E A L H N H Y T Q K S L S L S P
901 GGG AAA TGA
301 G K *

Figure 3

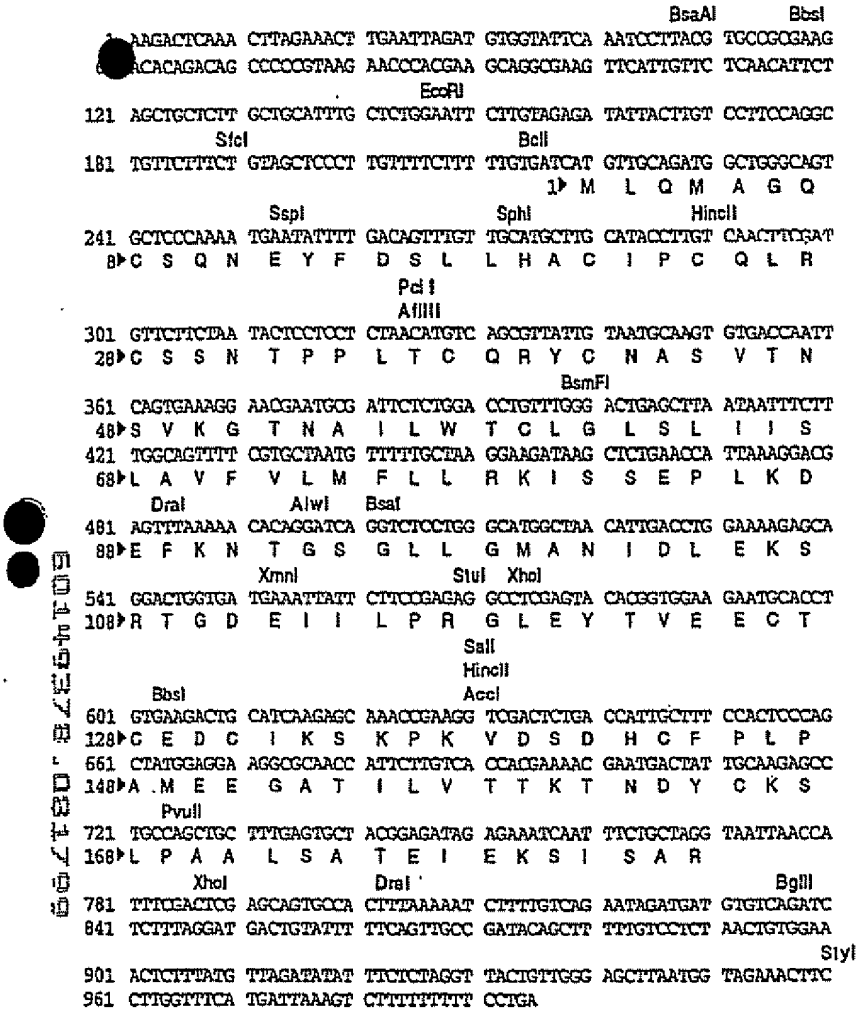
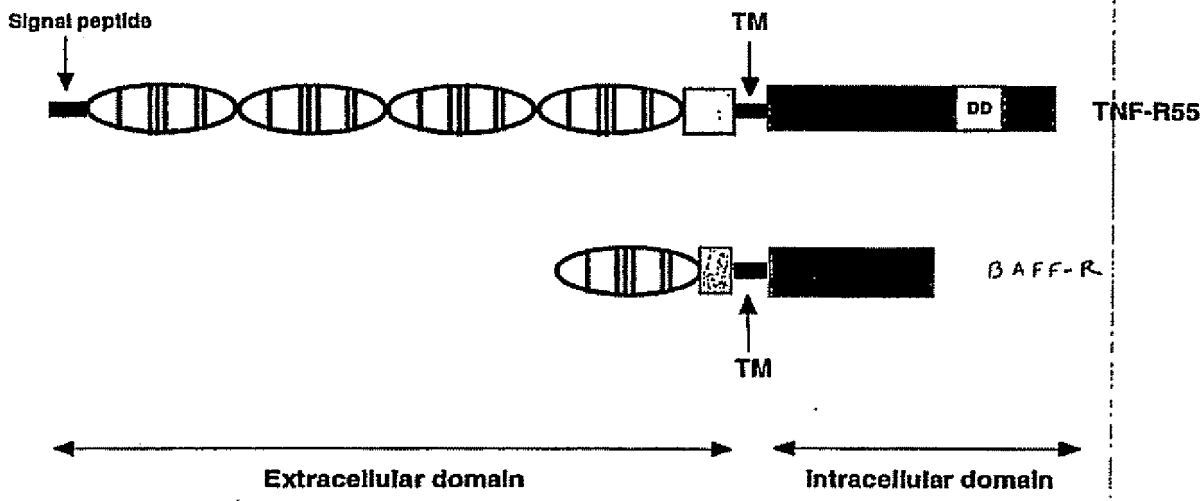

BsaAI
BbsI
EcoRI
SfcI
BclI
1▶ M L Q M A G Q
SspI
SphI
HincII
8▶C S Q N E Y F D S L L H A C I P C Q L R
PciI
AflIII
28▶C S S N T P P L T C Q R Y C N A S V T N
BsmFI
48▶S V K G T N A I L W T C L G L S L I I S
68▶L A V F V L M F L L R K I S S E P L K D
DraI
AlwI
BsaI
88▶E F K N T G S G L L G M A N I D L E K S
XmnI
StuI
XhoI
108▶R T G D E I I L P R G L E Y T V E E C T
SalI
HincII
AccI
BbsI
128▶C E D C I K S K P K V D S D H C F P L P
148▶A M E E G A T I L V T T K T N D Y C K S
PvuII
168▶L P A A L S A T E I E K S I S A R
XhoI
DraI
BglII
StyI

FIGURE 1

Structure comparison between TNF-R55 and BAFF-R



TM: transmembrane domain
DD: death domain

| Cysteine residues

Figure 5

A

B

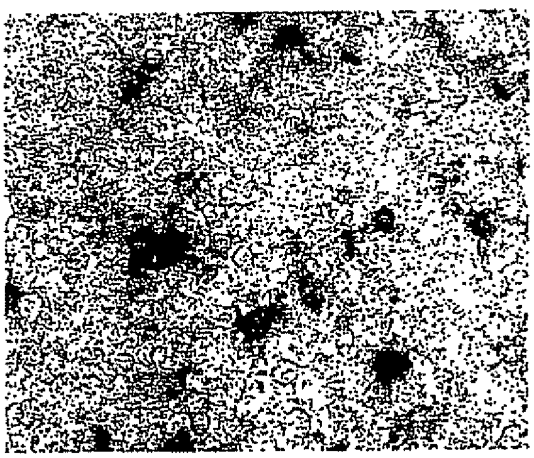


FIGURE 6(a)

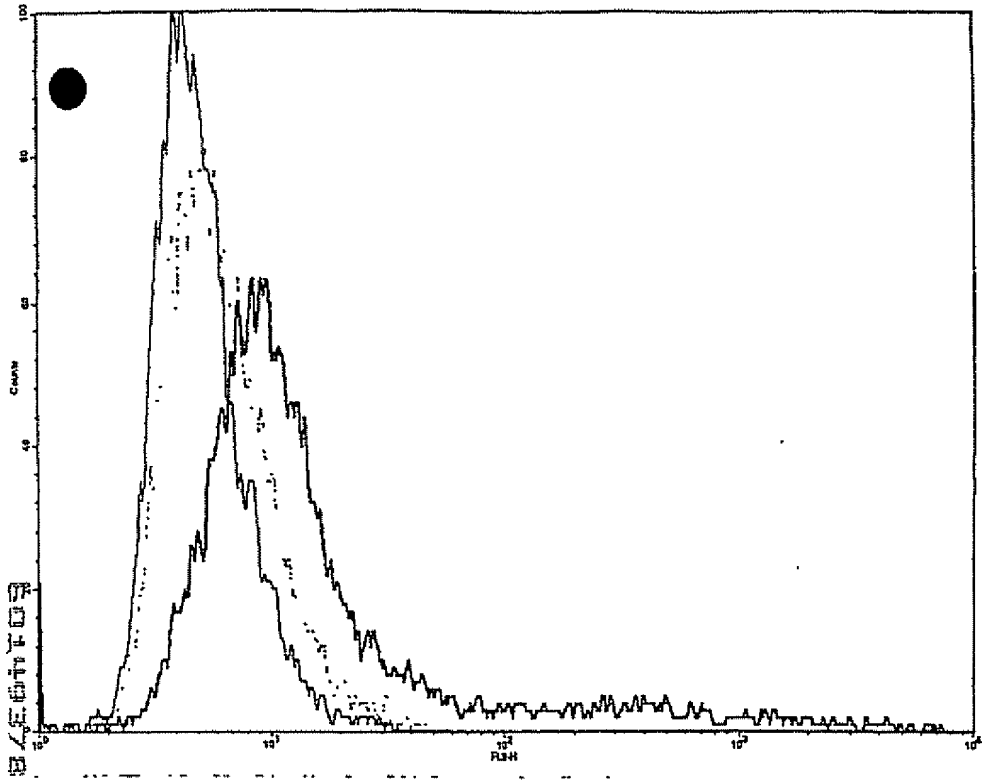


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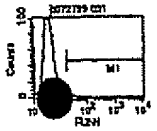
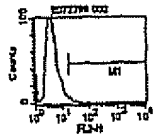
FIGURE 6B)



Histogram Statistics

File: p072799 001 — Log Data Units: Linear Values
Acquisition Date: 27-JUL-99 — Gate: No Gate
Gated Events: 10000 — Total Events: 10000
X Parameter: FL2-H (Log)

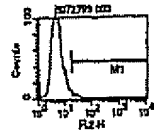
Marker	Left	Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1	9910	10000	100.00	100.00	4.28	3.82	81.34	3.63
M1	17	9910	65	0.65	0.65	23.23	22.44	30.37	20.25



Histogram Statistics

File: p072799 002 — Log Data Units: Linear Values
Acquisition Date: 27-JUL-99 — Gate: No Gate
Gated Events: 10000 — Total Events: 10000
X Parameter: FL2-H (Log)

Marker	Left	Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1	9910	10000	100.00	100.00	4.61	4.17	61.88	3.89
M1	17	9910	79	0.79	0.79	22.88	21.96	34.84	19.63



Histogram Statistics

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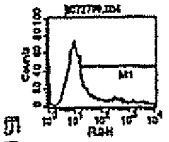
Acquisition Date: 27-JUL-99 — Gate: No Gate
Gated Events: 10000 — Total Events: 10000
X Parameter: FL2-H (Log)

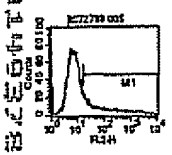
Marker	Left	Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1	9910	10000	100.00	100.00	5.61	4.93	58.41	4.68
M1	17	9910	133	1.33	1.33	23.65	22.06	29.53	22.57



Histogram Statistics

File: p072799 004 — Log Data Units: Linear Values
Acquisition Date: 27-JUL-99 — Gate: No Gate
Gated Events: 10000 — Total Events: 10000
X Parameter: FL2-H (Log)

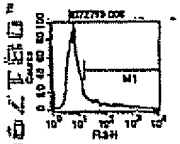
Marker	Left	Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1	9910	10000	100.00	100.00	108.34	13.40	439.27	10.27
M1	17	9910	2785	27.85	27.85	390.10	85.81	243.61	45.30



Histogram Statistics

File: p072799 005 — Log Data Units: Linear Values
Acquisition Date: 27-JUL-99 — Gate: No Gate
Gated Events: 10000 — Total Events: 10000
X Parameter: FL2-H (Log)

Marker	Left	Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1	9910	10000	100.00	100.00	72.63	11.42	518.47	7.84
M1	17	9910	2054	20.54	20.54	324.62	86.66	230.37	61.85



Histogram Statistics

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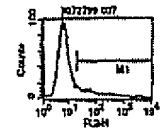
Gated Events: 10000 — Total Events: 10000
X Parameter: FL2-H (Log)

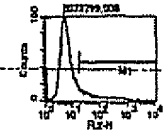
Marker	Left	Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1	9910	10000	100.00	100.00	51.15	9.41	568.93	6.67
M1	17	9910	1673	16.73	16.73	273.40	81.97	244.63	54.25



Histogram Statistics

File: p072799 007 — Log Data Units: Linear Values
Acquisition Date: 27-JUL-99 — Gate: No Gate
Gated Events: 10000 — Total Events: 10000
X Parameter: FL2-H (Log)

Marker	Left	Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1	9910	10000	100.00	100.00	29.59	7.74	578.84	5.94
M1	17	9910	1313	13.13	13.13	191.35	80.77	248.67	42.94



Histogram Statistics

File: p072799 008 — Log Data Units: Linear Values
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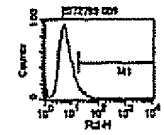
Gated Events: 10000 — Total Events: 10000
X Parameter: FL2-H (Log)

Marker	Left	Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1	9910	10000	100.00	100.00	12.39	6.54	405.32	5.47
M1	17	9910	876	8.76	8.76	73.84	43.41	153.84	33.63



Histogram Statistics

File: p072799 009 — Log Data Units: Linear Values
Acquisition Date: 27-JUL-99 — Gate: No Gate
Gated Events: 10000 — Total Events: 10000
X Parameter: FL2-H (Log)

Marker	Left	Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1	9910	10000	100.00	100.00	6.89	6.00	69.06	5.67
M1	17	9910	330	3.30	3.30	34.33	33.31	33.78	31.44

FIGURE 7